COUCH, J. N.

HETEROTHALLISM IN DICTYUCHUS, A GENUS OF THE WATER MOULDS.

[Annals of Botany, Vol. XL. No. CLX. October, 1926.]

[Printed in England.

Heterothallism in Dictyuchus, a Genus of the Water Moulds.[1]

BY

J. N. COUCH,

Botanical Department, University of North Carolina.

With Plates XXXV–XXXVIII and three Figures in the Text.

CONTENTS.

INTRODUCTION.

THE discovery by Blakeslee (1) of homo- and heterothallism in the Mucorineae opened up an entirely new field of research in the fungi. Since then investigations in some of the Basidiomycetes and Ascomycetes have shown that a similar sexual condition occurs in the species investigated of these two classes.[2]

In the Oomycetes no case of heterothallism supported by experimental evidence has yet been reported. The Saprolegniaceae especially have been favourite subjects for investigation by some of the most prominent botanists since early in the last century, and several species of this family have been

[1] Considerable work was done in preparing this paper for the press while the writer was working as a National Research Fellow in Botany.

[2] For bibliography on heterothallism in the Basidiomycetes see Hanna, W. F., The Problem of Sex in *Coprinus lagopus*, Ann. Bot., xxxix. 429–57, 1925. For a review of heterothallism see Cutting, E., Heterothallism and Similar Phenomena, New Phytol., xx, 1921.

[Annals of Botany, Vol. XL. No. CLX. October, 1926.]

reported to be dioecious. In no case, however, was this statement confirmed by experimentation.

In the present paper the first case of heterothallism in the Oomycetes is reported,[1] together with numerous experimental data.

It is a pleasure to acknowledge the invaluable criticism and encouragement given the writer during this investigation by Dr. W. C. Coker, under whose direction the work has been done. To Miss Alma Holland the writer wishes to extend his sincerest thanks for inking in Plates III and IV.

Historical.

Fifty-five years have elapsed since the genus *Dictyuchus* was founded by Leitgeb on the single species *D. monosporus*. During this period four new species have been proposed: Lindstedt in 1872 added the two species *D. Magnusii* and *D. polysporus*; Zopf in 1893 described as new *D. carpophorus*; and Coker in 1923 gave specific rank to a sexually sterile form, calling it *D. sterile*.

The genus itself, though very clearly separated from its nearest relatives, *Thraustotheca* and *Aplanes*, is nevertheless considerably confused as regards the species composing it, as can be clearly shown by a brief survey of the literature on the subject. Leitgeb (8) in describing the species *D. monosporus* says the sporangia are renewed as in *Achlya*, his figures also showing this condition; he describes the oogonia as being 25 μ thick, with antheridia which twine about them. Leitgeb also found a sterile species which bore only sporangia while under culture for four months. In *Dictyuchus Magnusii* the sporangia are described and figured by Lindstedt as being borne in rows; the oogonia are 30–35 μ thick, and the antheridial branches do not wind about the oogonia. Lindstedt, in the same publication, described *D. polysporus*, in which the vegetative growth and sporangia resembled *D. monosporus*, but with numerous eggs (2–20) in the oogonium, and with androgynous antheridia. These characters were so sharply in contrast with the single-egged oogonia and diclinous antheridia of the other two species that, taken together with the fact that the plant has not been found since first discovered, its validity as a species has been questioned by most subsequent observers, as Fischer (5), Minden (10), and Coker (4). It seems probable, as these authors suggest, that this species was no more than a sterile form of *Dictyuchus* mixed with an oogonia-bearing *Saprolegnia*. In 1893 Zopf (16) described as new a plant which he called *D. carpophorus*, the points of difference, according to his description, being in the peculiar lateral outgrowths of the hyphae, and in the fact that the oogonia, which contain

[1] First reported jointly by Dr. W. C. Coker and the writer before the Mycological Section of the Botanical Society of America, Cincinnati, Ohio, 1923.

a single eccentric egg, are often entirely encircled by antheridia which do not develop a fertilization tube. Coker (4), though not reducing any of the described species to synonymy, is of the opinion that *D. monosporus*, *D. Magnusii*, and *D. carpophorus* are the same. He is of the opinion, furthermore, that one of the two sterile plants reported by Humphrey (6) and the ones reported by Tiesenhausen (12), Minden (10), and Weston (15) are in all probability the same as his sterile species, *D. sterile*.

In addition to the confusion regarding species, there has also been a rather perplexing problem in regard to heterothallism in this genus. Leitgeb (8) and Lindstedt (9) both claimed that the antheridia and oogonia were borne on separate threads, and that their plants were therefore dioecious, a statement for which neither offers any cultural or experimental evidence. Neither Zopf (16) nor Humphrey mentions this matter. Weston (15) and Coker (4) suggest the possibility that the sterile form seen by them and others is either the antheridial or oogonial strain of a heterothallic plant.

Variability of Strains in Nature.

In the summer of 1923 an excellent growth of *Dictyuchus* was found growing on a lightning bug in Lake Mendota, Madison, Wisconsin. This culture was brought back to the laboratory at Madison and kept under observation for several days. The sporangia were formed as usual in great abundance, and their shape, size, and method of renewal agreed well with *D. sterile*, but a few oogonia were developed which resembled those of *D. monosporus* as illustrated by Coker. A species of *Dictyuchus* producing oogonia had never been observed by the writer before, and so considerable care was taken to secure a pure culture. A few healthy-looking threads were cut from the growth and transferred to a fresh Petri dish of corn-meal agar. The plant grew well on the agar, forming a good many sporangia; but no signs of any sexual organs were observed. Transfers were made to boiled seeds of various kinds, as hempseed, corn grains, radish seed, &c., and cultured in sterile lake-water. Cultures were also made in haemoglobin, leucin, levulose, with and without the addition of various salts as used by Kauffman (7), Pieters (11), &c. Cultures were also kept in the constant temperature room at about 14° C.; but none of these cultural methods was successful in oogonial production. Numerous collections were made from the lake to secure the fruiting stage of the plant again, but without success. It was suspected that the plant might be an oogonial or antheridial strain of a heterothallic species, and so it was brought back to Chapel Hill, North Carolina, with a view to crossing it with *D. sterile*.

The plant, as it has been growing under culture, agrees in all details with *D. sterile* of Coker (4), and so the following, with slight modification, is taken from him: Vegetative growth vigorous on hempseed, corn

grains, &c., extending out a cm. or slightly more from the substratum; hyphae 14–66 μ thick at base, usually about 30–48 μ, more or less branched according to the cultural conditions. Primary sporangia apical, the later ones formed by cymose branching, but usually separated from the earlier ones by some distance by the elongation of the threads. As the culture ages, the arrangement becomes more irregular and complicated, and most of the threads become segmented towards the periphery into numerous sporangia arranged in rows or branched groups. The sporangia are usually a little larger in the distal half, often bent, sometimes branched, of various size, 20–30 × 100–550 μ. They generally break off from the hyphae about the time the outline of the spores becomes distinct, and going into a resting state which may last a few days or many weeks, depending on conditions. During this time the spores are separated by walls which, in this condition, are scarcely visible, the individuality of the spores being indicated by the usually conspicuous vacuole that each contains. On emerging, the spores escape and swim, as is normal in the genus, or they often sprout in position into slender hyphae. Spores 11·8–16·6 μ in diameter, before sprouting, with a large conspicuous vacuole.

The Wisconsin strain, as mentioned above, was brought back to Chapel Hill with a view to crossing it with *D. sterile*. Numerous collections were made around Chapel Hill and, for the first time after fifteen years of collecting by Dr. Coker and his students, oogonia and antheridia appeared in three of the original lots of material brought in. The sexual organs appeared after the cultures had been in the laboratory about two weeks. One of these original lots of material, No. 1 of September 25, 1923, agreed best in most particulars with *D. Magnusii*, while the other, No. 1 of September 26, 1923, agreed best with *D. monosporus*. The former might be briefly characterized as follows: Hyphae stout, vigorous (on hemp-seed) as in the *Achlya racemosa* group, considerably branched. Sporangia usually long-cylindric, rarely short-clavate or oval, mostly 300–400 μ long, not rarely up to 1 mm. long or slightly longer. Spores are characteristic of the genus. Oogonia spherical or slightly subspherical, 35–52 μ thick, wall smooth, unpitted. Eggs single, 30–34 μ thick, mature eggs eccentric. Antheridial branches of diclinous origin; antheridia large, conspicuous, usually one or several on each oogonium, not rarely nearly completely wrapping around the oogonium, sometimes absent from an oogonium, in which case the oogonium may form a parthenogenetic egg. Antheridial tubes developed and clearly visible.

The present plant agrees well enough with *D. Magnusii*, as originally described by Lindstedt, in the size of the oogonia and eggs, but differs from that plant in the fact that the sporangia here are almost always borne in sympodia, while Lindstedt describes the sporangia in his plant as being in rows. The number of antheridia on the oogonia and their mode of

application are quite variable: the number varying from one to several, which may be applied laterally, or may completely encircle the oogonia. In order to distinguish this strain from the others it has been designated 'C'.

The second lot of material (collection No. 1 of September 26, 1923) contained a plant which agreed with the above in vegetative growth and sporangial characters, but differed from it in the size of the oogonia and eggs. The oogonia of the latter species measured 27–33 μ thick, the eggs 25–29 μ, eccentric, thus agreeing in this respect with *D. monosporus*. The antheridia varied from 1 to 5 on the oogonia, and might be tuberous and applied by their ends, or might be elongated finger-like and completely encircling the oogonia. The two plants could hardly be distinguished on the antheridial characters. This strain was designated 'B'.

A third lot of material was collected which contained antheridia and oogonia in the original culture. This plant might be characterized briefly as follows: Hyphae up to 1 cm. long, varying considerably in thickness. First sporangia formed in sympodia, the later ones formed in a row, sometimes as many as ten sporangia in a single row, quite variable in size and length, 12–38 × 100–200 μ, spores sometimes in a single row, sporangia deciduous. Oogonia 27–33 μ thick, eggs 22–29 μ, eccentric. Antheridia wrapped about the oogonia.

This plant agrees best with the description of *D. monosporus* and with strain 'B' above. It is noteworthy that the sporangia of this plant agree perfectly with those of *D. sterile*, and combine the characters of *D. monosporus* and *D. Magnusii*. This strain has been designated 'I'.

Numerous other sterile strains have appeared around Chapel Hill which, with but two exceptions, when grown unmixed with other strains of the opposite sex, agree with *D. sterile*. The thallic character of these strains as revealed by contrasting them with the oogonial and antheridial strains will be discussed at some length later on in this paper. One of the plants mentioned above which did not agree with *D. sterile* differed from it in that it combined the sporangial characters of *Dictyuchus* and *Thrausthotheca*. The original culture contained many sporangia, the walls of which were bursting open and going to pieces, much as in *Thraustotheca*, and thus liberating the encysted spores. On the same threads with the *Thraustotheca*-like sporangia there could quite often be found sporangia of the *Dictyuchus* type. This strain has been designated 'G'.

Four other strains of *Dictyuchus* were collected during the last week in April, 1924, from near Georgetown, S.C., on the coast. Three of these strains, designated 'J', 'K', and 'L', differed in no particular from *D. sterile*. The other strain, designated 'M', which was bearing a large number of oogonia, was found growing on a mat of dying *Spirogyra* which had been parasitized by *Aphanomyces phycophilus*. The early sporangia of 'M'

differed in no way from those found in the original culture of strain B or C. The later ones, however, were quite irregular in shape, quite often antheridial threads or even antheridia being converted into sporangia, resembling very closely Zopf's figures of the sporangia of *D. carpophorus*. The oogonia were borne as in strain B and varied in size from 22 to 37 μ, the average of ten measurements being 30·3 μ, a good many proliferating and then without eggs, the eggs 18–32 μ, average of ten measurements 23 μ, eccentric. Antheridia on all oogonia, which contained an egg, simple or coiled around the oogonia.

During the latter part of June, 1924, the writer collected three strains of *Dictyuchus* in Florida. Two of these, designated 'O' and 'P', resembled *D. sterile* when grown separated from other strains. Their thallic character will be discussed later.

The other strain, designated 'N', collected in Florida differed from the other described strains in that it formed oogonia and eggs without any antheridia. The plant may be described as follows:

Vegetative growth and asexual reproduction as in *D. monosporus*, *D. sterile*, &c. Oogonia borne usually on threads which are considerably thinner and shorter than the main hyphae, and usually formed on the bottom of the culture, and hence often hard to see unless the culture is turned over; oogonial stalks commonly curved and often bearing several oogonia, 27–44 μ thick, most 35–39 μ; usually spherical but sometimes slightly subspherical, smooth, and without pits. Eggs 23–35, most 29–33 μ thick, spherical, single in the oogonium, eccentric; antheridia not developed when the fungus is grown separate from other molds.

The present strain is easily distinguished from the others by its peculiar habit of forming oogonia and eggs without antheridia. The fact that the oogonia are borne on more or less specialized branches which usually grow out on the under side of the culture will also help to distinguish this plant. The oogonia, though formed in nearly all cultures, appear rather late, not before the fourth or fifth day, and never in large numbers. Some cultures, though quite vigorous and healthy, may bear only a single oogonial thread which in turn bears several oogonia. The results of crosses between this plant and other strains will be discussed farther on in this paper.

An effort was made to increase oogonial production by selecting the oogonial threads and making subcultures from them. This selection was carried only through a few generations; the cultures, however, showed no increase in oogonial production. Cultures on hempseed to which were added the solutions recommended by Kauffman (7) and Pieters (11) for oogonial production were made, but without any perceptible increase in the number of oogonia.[1]

[1] The writer has collected eight strains of *Dictyuchus* from different localities on Long Island, N.Y. All of these agree with *D. monosporus*, *D. sterile*, &c., in vegetative and asexual characters.

Crosses to determine the Thallic Character and Distribution of the Strains in Nature.

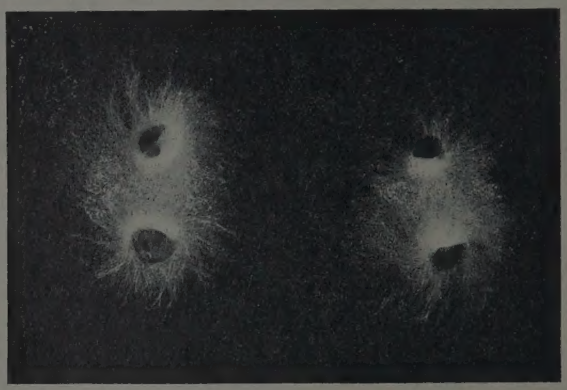
The original culture of strain 'B' contained an excellent lot of oogonia and antheridia. It was noticed that threads which bore oogonia never bore antheridia, and vice versa. But the oogonial threads occasionally bore at or near their tips sporangia, as did also the antheridial threads. It was hoped that by separating what seemed to be male and female parts and growing them alone, and then contrasting them on suitable media, the question as to whether the plant was heterothallic or not could be finally settled; and so a sporangium from an oogonial thread was carefully teased out under a low-power binocular with sharp needles and transferred to a corn-meal agar plate. This was also done with a sporangium from an antheridial thread. The strain descended from the sporangium cut from the oogonial hypha grew well on the corn-meal agar, forming sporangia but no oogonia. Cultures were made on oat-meal agar, small bits of hempseed, mushroom grubs, and on termites in sterile well-water, none of which, though the cultures were repeated many times, produced any oogonia, while sporangia were formed in great abundance. The same results were obtained with the strain descended from the antheridial sporangium. In the vegetative growth and sexual reproduction the strain descended from the female sporangium was indistinguishable from that descended from the male sporangium, there being no difference in vigour of growth as was described by Blakeslee (1) in the opposite strains in some of the heterothallic Mucorineae.

Unsuccessful in these attempts to induce the formation of oogonia by alteration in the nutritive medium, the male and female strains were grown together in the same dish with the mycelium of each strain partly in contact with that of the other. The methods pursued which gave the most satisfactory results were about as follows: Corn-meal agar cubes about 2–3 mm. thick were cut from the periphery of the circular area where the antheridial strain of the fungus was growing, so as to include in each of the cubes a good supply of the end region of the growing threads. Several of these cubes were placed in a sterile Petri dish, separated from each other and from the sides of the dish as far as possible. On each cube was placed a small piece of boiled hempseed (usually a half hempseed to each piece). To each of the incipient cultures was added a few drops of water. When the cultures were 24–48 hours old they were carefully washed by squirting a small stream of sterile well-water over them, and then fresh water was

Seven of them have, so far, given only neutral reactions with the stock male and female strains; the eighth is homothallic, but agrees in all other respects with the heterothallic strains. Detailed studies on these strains will be reported in a subsequent paper.

added. Cultures of the oogonial strain were made at the same time and in the same way, but were, of course, kept in separate dishes. When the cultures were about three days old an antheridial culture and an oogonial culture were transferred to a fresh Petri dish and placed so that the threads of the two strains were in contact. These cultures, unless otherwise noted, were kept at room temperature.

Two such crosses were made on October 20 between the antheridial and oogonial strain of B. These were examined three days later, and in the region where the threads of the two different strains were in contact



TEXT-FIG. 1. Natural size photograph showing two contrasts between male and female strains of *Dictyuchus*. The white specks in the regions where the threads intermingle are the sex organs. The elongated white specks scattered over the figure are sporangia which have become separated from the threads.

many oogonia and antheridia were formed, as is shown in the accompanying photograph (Text-fig. 1), while in the area where the threads were not in contact none was observed. Out on the margin of the region in contact where the hyphae were not so numerous and dense, the oogonial and antheridial threads could be traced back to the pieces of hempseed inoculated from the oogonial and antheridial strains respectively. Numerous contrasts were made in the same way as those above, except that an antheridial strain was contrasted with an antheridial and an oogonial with an oogonial, but in no such contrast were any oogonia or antheridia formed. From these two original oogonial and antheridial sporangia over two hundred cultures have now been made, and in none of these have any oogonia or antheridia appeared so long as they have been kept unmixed with the strain of the opposite sex. Over a hundred crosses have been made with these two strains, and almost invariably, when the cultures were young, healthy, and free from bacteria, oogonia and antheridia were formed in the region where the threads of the two opposite strains were in contact.

It is a noteworthy fact that in the later crosses between these strains the sexual reaction has become weaker; at present (March 1, 1924), oogonia are formed rather sparingly in the crosses and sometimes not at all. This gradual diminution in the ability to form sexual bodies was also found by Blakeslee (1) in a strain of *Mucor mucedo* when the cultures of the fungus were made from the mycelium.

In several of the crosses a rather peculiar and interesting variation appeared. The antheridia, which were quite variable even in the original lot of material, in these crosses, notably No. 45, were in many cases very elaborately developed, forming quite often a network around the oogonia several layers of threads thick. Even in these cases, however, one to several antheridia were cut off, antheridial tubes were developed, and fertilization apparently took place. These elaborately developed antheridial branches, twining about the oogonia so as to form a hull or network around them, were very suggestive of a similar condition described by Zopf in *D. carpophorus*, but his figures do not show the condition nearly so elaborate as it may be. In spite of Zopf's extensive description of his plant, some of the most critical diagnostic points, such as the size of the oogonia and eggs, are omitted, but from the facts at hand it seems probable that his plant is a variation of ours.

An effort was made to separate the two sexes in strain C, but unfortunately the male sporangium failed to grow.

In strain I the two sexes were successfully isolated in the same manner as described above for strain B. The first crosses were made on February 20. In five of these crosses both strains were growing on hempseed, in one the antheridial strain was on corn and the oogonial on hempseed, in two both strains were on corn. These were examined on February 22, and only in the two crosses on corn were any oogonia and antheridia formed. These crosses were examined again on February 29, but without any noteworthy change having taken place since the 22nd. Later crosses were made, but gave no sexual reaction at all. It seems that either the present strain is capable of giving only a weak sexual reaction, or that the proper environmental conditions for the production of oogonia and antheridia have not yet been afforded the plant.

The two sexual strains were isolated in strain M in the same manner as described above for strain B. The strains were cultured alone without the formation of any sexual organs, but when the two strains were grown in contact oogonia and antheridia were formed.

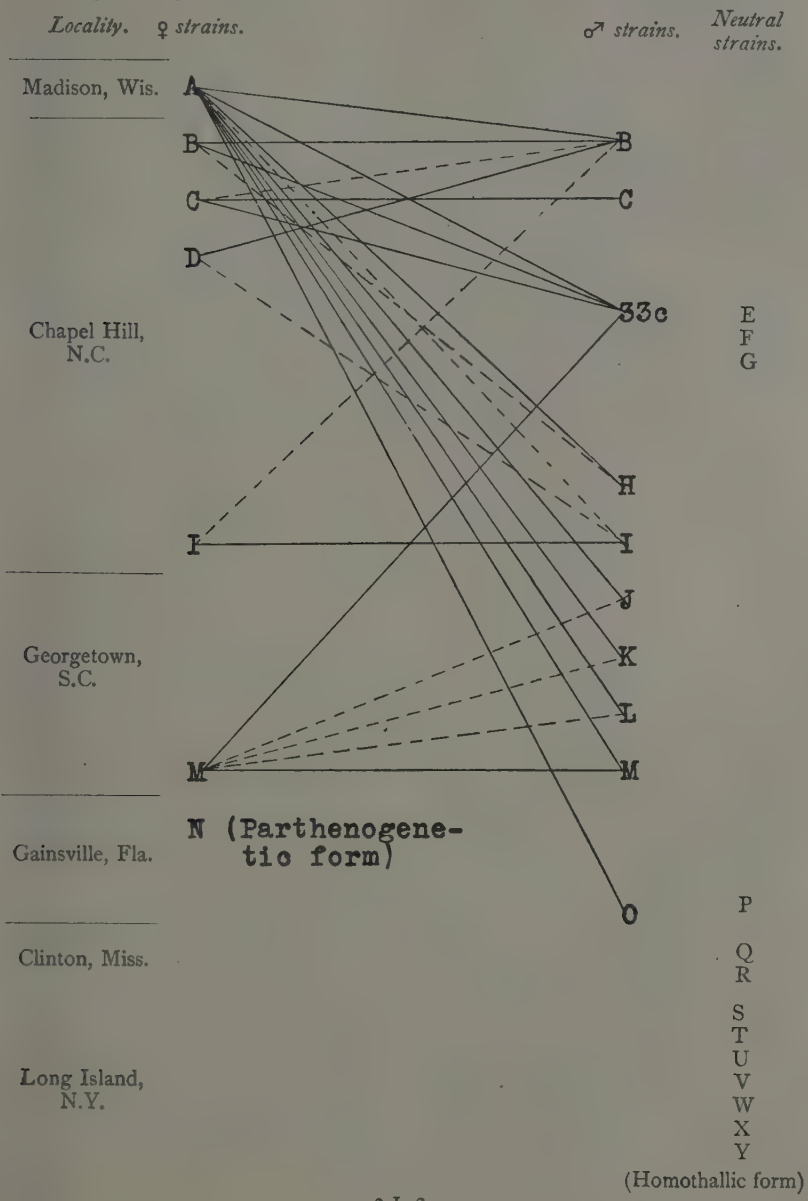
The results obtained by intercrossing the several strains brought to light some strikingly interesting information (see Table I). The first intercrossing was made with strain A from Wisconsin and both sexes of strain B from Chapel Hill. Numerous crosses were made between strain A and strain B ♀, the cultures being grown on a considerable variety of media, as

pieces of boiled hempseed, corn grains, mushroom grubs, ant larvae, &c. Crosses were also kept in the oven with the temperature around 26° C. In none of the crosses, however, were any oogonia or antheridia produced. A healthy young culture of strain A, growing on a piece of hempseed, was crossed on November 1 with strain B ♂ growing on a piece of corn grain. The two cultures were placed so that the hyphal threads of each culture were in contact a short distance along the margin with the other. In this cross, three days later, a great many oogonia and antheridia were forming in the region where the threads were in contact. The oogonia were borne on threads which could be traced back to the hempseed, and the antheridial branches could be traced back to the corn grain. A good many oogonia were seen without antheridia, and in such cases failing to mature eggs. Over fifty crosses between strain A and strain B ♂ have now been made, and without exception, when the cultures were fairly free from bacteria, oogonia and antheridia were formed. The oogonial nature of strain A was thus established.

A comparison of the crosses between B ♀ and B ♂ and A ♀ and B ♂ reveals some interesting facts regarding the relative vigour of the oogonial strains in their reactions to the antheridial strain. As has already been noted above, in the first crosses between B ♀ and B ♂ many oogonia were produced, and in some of these crosses the antheridial branches were very elaborately developed. Gradually, however, the two strains seemed to lose their sexual vigour, and as the crosses were made fewer and fewer oogonia and antheridia were produced, until the present time (spring, 1924) only a small percentage of the crosses have any sexual organs, and these are sparingly formed. In the crosses between B ♂ and A ♀ the sexual reaction was very strong at first, the oogonia being formed before the antheridial branches became obvious, and in such abundance that many of the oogonia were not furnished with antheridia, and in such cases failed to mature eggs. The oogonial strain A seemed to be much more strongly stimulated than the antheridial, whereas in the crosses between B ♀ and B ♂ the antheridial strain seemed to be the one most stimulated.

Other experiments which indicate in a much more striking manner the relative strength of the oogonial strains when crossed with antheridial strain B were carried out. In one of these crosses the three strains were placed together in the same dish, and were arranged so that strain B ♂ was in the centre with strain B ♀ in contact on one side and strain A ♀ in contact on the opposite side. All the cultures were growing on hempseed, and were young, healthy, and practically free from bacteria. The crosses were examined eight days later, and many oogonia and antheridia were formed between A ♀ and B ♂, but none was formed between B ♀ and B ♂. Upon examination four days later, a few oogonia and antheridia were formed between B ♀ and B ♂. These crosses were repeated several times with

TABLE I. *Showing the Total Number of Female, Male, and Neutral Strains collected in Nature. The strains that crossed with each other are joined by a solid line. Sexual strains between which no reaction was obtained are joined by a dotted line.*



similar results, except that in a few of the crosses no sexual organs at all were formed between the strains B ♀ and B ♂. In another set of crosses the three strains were grown together in Petri dishes on corn-meal agar, made up according to the following formula: 3 grm. of agar shreds plus the filtered decoction from 20 grm. of corn-meal soaked in 500 c.c. of lukewarm water one hour; sterilized under 15 lb. pressure for thirty minutes in the autoclave. The agar plate was inoculated in the following manner: healthy threads were cut from a hempseed culture of strain B ♂ and placed approximately in a straight line in the centre of the dish, while threads were cut from strain A ♀ and B ♀ and placed on the plate in line with the threads of strain B ♂, but on opposite sides of the latter. The threads of the three strains were placed so that there was 0·8–10 mm. space between each line. The culture was examined two weeks after being made, and many oogonia and antheridia were formed between strain A ♀ and strain B ♂, but none was formed between strain B ♀ and B ♂. Similar crosses were made with practically the same results. The approximate sexual strength of the several strains may be judged from the data summarized in Table I.

It remained now to test the reaction of strain C, strains I ♀ and I ♂, and several other strains apparently identical with *D. sterile* with strain A ♀ and B ♂ and B ♀.

Numerous crosses were made between A ♀ and C, B ♂ and C, and B ♀ and C on different kinds of media and under different temperatures, but without the formation of any sexual bodies. Later, however, strain C was crossed with a strain 33 c ♂ (the origin of which will be explained farther on). This cross was made on February 11. Upon examination six days later, no sexual bodies had been formed. The culture was put in the oven at this time and examined again on February 22, at which time many oogonia and antheridia were formed. The thickness of ten oogonia, measured as they came into the field, varied from 34 to 48 μ, five of them being 37 μ thick; the ten eggs in these oogonia varied from 27 to 33 μ, five of them being 29 μ thick. There were one to several antheridia on each oogonium, at times completely covering the oogonium, in other cases applied only for a short distance along the oogonial wall. The oogonial nature of strain C was thus established, and also the identity of the plant which had been at first called *D. Magnusii* (strain C) with *D. monosporus* (strain B).

Several strains had been collected which in vegetative growth, sporangial characters, and the absence of sexual reproductive organs agreed with *D. sterile*. These were now crossed with the male and female strains to test their sexual reaction. Strain D, collected from the Arboretum Branch, January 10, and purified by the isolation of a single thread, was crossed on January 26 with strain A ♀, strain B ♀, and B ♂. Upon examination, January 29, no oogonia were observed. Strain B ♂, however, was branching considerably, forming what appeared to be antheridial branches. The cross

was examined again on February 7, but no oogonia had been formed. At this time the cultures were put in the oven, where the temperature was 26° C. Upon examination, four days later, a few oogonia had been formed between strain B ♂ and D. Several other similar crosses were made at the same time, and underwent the same conditions without the formation of any oogonia. It was suspected, therefore, that strain D was a mixed lot, partly neutral and partly weakly oogonial in nature, and an effort was now made to isolate the oogonial strain from the neutral strain. To do this, two sporangia from an oogonial thread were separated out and transferred to a corn-meal agar plate. Cultures descended from these sporangia were then made, and were contrasted on February 11 in the usual manner with the strain descended from sprouting egg 33 c, a very strong male strain. Upon examination, February 17, no oogonia were formed. The culture was then put in the oven, where the temperature was about 26° C.; it was examined again on February 29, but no oogonia had been formed. This cross was repeated several times with similar results. Crosses were then made with strain I ♂ and also with other strains, as strains G and F, but without the production of any sexual organs.

Strain E, also collected from the Arboretum Branch on January 10, was put through the same series of crosses as D, but without the formation of any sexual organs.

Strain F from Battle's Branch, January 16, which differed slightly from typical *D. sterile* in having considerably coarser hyphal threads and longer and larger sporangia, was crossed with strain A ♀ and B ♂, as described above, but without the appearance of any sexual organs. A cross on corn-meal agar was made in which threads of strain F were placed about the centre of the dish, with threads of strain A ♀ on one side and B ♀ on the other and B ♂ at one end, the three latter being arranged so that their threads would come in contact with those of strain F before they came in contact with each other. The threads of the latter three strains, however, showed an excellent growth in the direction away from F, but grew very poorly towards F, never coming in contact with F. Strain F grew very poorly itself except at the end away from strain B ♂, where its threads had a free range to the edge of the dish. Whether to consider strain F as a neutral strain or as a male or female strain of another species is a matter which can hardly be decided at present. It seems, however, that since both the antheridial and oogonial strains of *D. monosporus* are repelled by strain F the latter view is more probably correct.

A very peculiar strain G of *Dictyuchus* was collected from Howell's Branch on January 10. The original culture contained many sporangia, the walls of which were bursting open and going to pieces much as in *Thraustotheca*. The culture was purified by isolating on an agar plate a single sporangium of the *Thraustotheca* type from which the strain is descended.

Numerous contrasts were made in which antheridial strain B and an antheridial strain designated 116 (descended from a sprouting egg which was taken from cross No. 116 between B♀ and B♂) and strain A♀ and B♀ were used. In none of the crosses, however, was there any sign of oogonia.

Strains 'J', 'K', and 'L' from Georgetown, S.C., when unmixed with other strains, resembled *D. sterile*, were crossed with the stock male and female strains, but gave a reaction only with the female strain, thus proving them to be male in nature.

Strain 'O' from Florida was contrasted several times with the stock oogonial (A) and antheridial (33 c) strains, giving a strong reaction with the stock female strain, but none with the stock male strain, thus showing strain 'O' to be antheridial in nature. Strain 'P' from Florida gave no reaction in contrasts with either male or female strains.

Crosses were also made between the male and female strain of M and A♀ and 33 c ♂. The strain descended from the male sporangium designated M was crossed with A♀, many oogonia, eggs, and antheridia being produced. The oogonia varied in size from 37 to 55 μ, the average of twelve measurements being 43 μ thick; the eggs 29–44 μ, the average of the same number of measurements being 34 μ. A cross between the strain descended from the female sporangium M and strain 33 c ♂ produced oogonia, eggs, and antheridia much as in the above cross, but not in such abundance. Crosses between M♀ and M ♂ also gave a sexual reaction, as has been noted above, though it was considerably weaker than in either of the above crosses and the eggs failed to mature. It is interesting to note the remarkable variation in the size of the oogonia and eggs in the original culture of strain M as compared with the size in the cross with A♀ as shown in Table II.

In crosses which exhibited such remarkable variation in sexual activity one would reasonably expect considerable variation in the size of the oogonia and eggs, and also in the number of antheridia. As a matter of fact the oogonia and eggs showed considerable variation, but this seemed to be effected not only by contrasting a different oogonial strain with the antheridial, but also by altering the substratum on which the fungus was growing. The results in the following crosses represented in Table II will show the variation in the size of the oogonia and eggs. The oogonia in the above crosses show a total variation in thickness from 22 to 55 μ, the eggs from 18 to 44 μ, a variation certainly wide enough to discredit the validity of the two species *D. monosporus* and *D. Magnusii*. The antheridia varied from a simple, single, tuberous antheridium to a very elaborate network of threads completely surrounding the oogonium. The wide variations in the antheridia were quite difficult to explain, the variations occurring quite often in the same culture under apparently the same external conditions.

From the crosses described above it seems quite logical to conclude that *D. monosporus* and *D. Magnusii* are simply variations of the same species;

as is also, in all probability, *D. carpophorus*. The other forms which so far have failed to give any reaction with either the antheridial or oogonial strains may be unisexual or neutral strains of one or more entirely different species, or they may be neutral strains of *D. monosporus*.

TABLE II.

Variation in Size of Oogonia and Eggs in Different Strains and Crosses.

Strains crossed.	*Medium used.*	*Range in size of oogonia.*	*Average size of 10 oogonia.*	*Range in size of eggs.*	*Average size of 10 eggs.*
(1) Strain B, original culture	On mushroom grub in H_2O, room T	27–33 μ		25–29 μ	
(2) Strain C, original culture	On corn grain in H_2O, room T	35–52 μ		30–34 μ	
(3) Strain I, original culture	On corn grain in H_2O, room T	27–33 μ		22–29 μ	
(4) Strain M, original culture	On dying spirogyra in H_2O, room T, 22–24° C.	22–37 μ	30·3 μ	18–32 μ	23 μ
(5) B♀ × B♂	On hempseed in H_2O, room T, 20–22° C.	32–44 μ	37 μ	25–34 μ	29 μ
(6) A♀ × B♂	On hempseed in H_2O, room T, 20–22° C.	35–48 μ	40 μ	28–37 μ	33 μ
(7) A♀ × B♂	On hemp seed in H_2O, room T, 26° C.	33–55 μ	42 μ	25–40 μ	33 μ
(8) A♀ × B♂	On corn-meal agar, room T, 20–22° C.	29–40 μ	33 μ	22–25 μ	23 μ
(9) C♀ × 33 c♂	On hempseed in H_2O, room T, 26° C.	34–48 μ	35 μ	27–33 μ	29 μ
(10) A♀ × M♂	On hempseed in H_2O, room T, 22–24° C.	37–55 μ	43 μ	29–44 μ	34 μ
	Total range,	22–55 μ		18–44 μ	

Numerous crosses have been made between the parthenogenetic strain and the male and female strains of *D. monosporus*. In twelve crosses between cultures descended from single spores from the parthenogenetic strain and strain A♀ no reaction took place, the parthenogenetic strain forming oogonia and eggs as usual, while the female strain of *D. monosporus* was unaffected. Twelve crosses were also made between the spore strains and 33c♂. In each case two cultures of the parthenogenetic strain were put in the dish, one in contact with the antheridial strain, the other one not in contact. On all the cultures of the parthenogenetic strain oogonia were formed as usual, and in one of the crosses a few antheridia were formed by 33 c♂ and were wrapped around the *Dictyuchus* oogonia.

In view of the fact that the parthenogenetic strain so closely resembled the other strains of *Dictyuchus* it seemed a matter of interest to germinate

the parthenogenetic eggs in the hope that the strains from the germinated eggs might throw some heterothallic varieties.

During the first part of January, 1925, four eggs were successfully isolated and germinated. A description of the methods used in egg germination is given farther on in this paper. These eggs were designated W, X, Y, and Z. The mycelium from sprouting egg W was divided into forty-nine parts, and each part was planted separately on a corn-meal agar plate; while the mycelia from sprouting eggs X, Y, and Z were transferred in entirety to separate corn-meal agar plates. After considerable growth had taken place, six cultures were made on hempseed in water from each of the forty-nine parts of W, and six each from X, Y, and Z.

Two contrasts were now made between each of the fifty-two strains (W 1–49, X, Y, and Z) and 33 c ♂ and A ♀.

In eighty-eight of the ninety-eight contrasts with 33 c ♂ and W 1–49 oogonia were formed on the strains descended from the parthenogenetic form, as usual when growing alone. In the remaining ten, no oogonia were seen on the parthenogenetic form, a fact of significance, however, only when these results are compared with the contrasts with A ♀, since some of the cultures of the parthenogenetic strain, when grown alone, form no oogonia. In sixteen of the ninety-eight contrasts the male strain, 33 c, was stimulated to the formation of antheridia which applied themselves to the walls of the oogonia of the parthenogenetic strain.

In each of the ninety-eight contrasts with A ♀ oogonia were formed on the cultures of W 1–49; in six of the contrasts very many oogonia were formed on W; in eight very few oogonia (not more than a dozen in each culture) were formed on W; while in the remainder from a few to a considerable number were formed on W. In two of the contrasts quite remarkable results were obtained. In these two not only did the parthenogenetic strain form oogonia, but A ♀ formed them also, and in many cases the latter were furnished with antheridia arising from the parthenogenetic strain, in which cases, as a rule, eggs were formed.

In the contrasts between eggs X, Y, and Z and 33 c ♂ oogonia were formed on the cultures of X and Z, but none were formed on the cultures of Y. No antheridia were seen.

In the contrasts between eggs X, Y, and Z and A ♀ oogonia were formed on all the cultures of the parthenogenetic strain, and in the contrasts between Y and A ♀ oogonia were formed on the latter and were furnished with antheridia which came from Y.

These experiments, though not succeeding in bringing out any heterothallic varieties of the parthenogenetic form, showed that the antheridial nature, though normally latent in the parthenogenetic form, was present nevertheless and could be brought out under certain conditions.

Crosses with other Moulds.

Blakeslee (1), in his work with the Mucorineae, was able to induce a process of imperfect hybridization by contrasting opposite strains of different heterothallic species in the same or even in different genera. It seemed a matter of considerable interest to make similar tests with the antheridial and oogonial strains of *Dictyuchus*. So far, contrasts have been made only with the genus *Thraustotheca*, the two species *T. clavata* and *T. primoachlya* being used with plus and minus strains of *Rhizopus* and *Phycomyces*. The two species of *Thraustotheca*, because of their close relation to *Dictyuchus*, offered especially good subjects for experimental crosses. Both species are homothallic plants, the antheridia of *T. clavata* being of diclinous origin while those of *T. primoachlya* are of androgynous origin. When well nourished, both plants produce abundant oogonia and antheridia in from three to five days. Several crosses have been made between *T. clavata* and strain A ♀ and B ♂, but without any apparent sexual stimulation on the part of either plant. The crosses between *T. primoachlya* produced considerable sexual stimulation. On February 12 a healthy two-day-old culture of *T. primoachlya* growing on a piece of hempseed in water was crossed with strain A ♀ and B ♂, the two strains of *Dictyuchus* being put on opposite sides of the culture of *Thraustotheca* so that there would be no chance of the two strains of *Dictyuchus* crossing with each other. The cross was examined four days later. The *Thraustotheca* had formed many oogonia over the entire culture, and many elaborately branched antheridial threads which, however, were much more elaborately formed on the side with strain A ♀ than on the opposite side. Between strain A ♀ and the *Thraustotheca* a few *Dictyuchus* oogonia were formed, which were borne on much-coiled and sometimes elaborately branched threads. None of the *Thraustotheca* antheridia were applied to the oogonia, which, as a probable consequence, failed to form any eggs. Between the strain B ♂ and the *Thraustotheca* there was no reaction. The culture was examined again a week later: the *Thraustotheca* had practically exhausted itself in the formation of sporangia and oogonia, but the two strains of *Dictyuchus* were still healthy and growing. And from some of the threads of strain B ♂ delicate antheridial threads had grown, extending across the *Thraustotheca* culture to apply themselves to oogonia formed by strain A ♀. It was a matter of considerable importance to determine whether the *Thraustotheca* had caused the stimulus in the two strains of *Dictyuchus*, or whether in some way the stimulus from the antheridial to the oogonial strain and vice versa had passed through the *Thraustotheca* threads. Accordingly the *Thraustotheca* was crossed with strain B ♂ alone on February 19. The culture was examined on February 21, but there was no obvious sexual reaction, nor was there any on February 25 or 29 or March 3. Two crosses of *Thraustotheca* with strain

A♀ were made at the same time as the one described above. On February 21 *Dictyuchus* oogonia were forming in the region where the threads of the two fungi were in contact. The antheridial branches of the *Thraustotheca* were considerably stimulated on the side next to the *Dictyuchus*, but none was applied to the oogonia of the latter. Upon re-examining on February 25, 29, and March 3 none of the *Dictyuchus* oogonia was seen to be maturing eggs. The second cross was much as the above except that some *Thraustotheca* antheridia were apparently applied to the *Dictyuchus* oogonia. No eggs, however, matured in this cross.

All possible crosses were also made between the different sexual strains of *Dictyuchus* and plus and minus *Rhizopus* and plus and minus *Phycomyces*.[1] With these latter crosses corn-meal agar was used, a medium which would seem particularly adapted to these contrasts, since the opposite strains of *Rhizopus*, *Phycomyces*, and *Dictyuchus*, when crossed on it, produced abundant sexual reproductive bodies. One set of crosses was made on February 15 and kept out in room temperature. Several later examinations revealed no sexual response between the threads. It was interesting to note that in all the crosses the threads of the two fungi did not repel each other but intermingled. Another set of crosses was made on March 5 and kept in the incubator, where the temperature was about 26° C., with the same results as those just described above. It is quite possible, in spite of the above results, that a sexual response may be induced between strains of certain species of the Mucorineae and *Dictyuchus*, and it is hoped that a more exhaustive investigation may be carried out later.

Egg Germinations and Sex Differentiation.

The differentiation of this heterothallic species of *Dictyuchus* into antheridial and oogonial strains which, when cultured separately, are indistinguishable from each other, but which when crossed, even after the thirtieth non-sexual generation, produce sexual organs of such marked morphological distinctness as the antheridia and oogonia, offers a very striking example of heterothallism, for here in the fruiting condition the distinction between male and female is obvious, while in the Mucors no such distinction is possible, as Blakeslee (1) has shown. In *Dictyuchus* the germinating egg normally gives rise to a mycelium which may form one or several sporangia, and these in turn bear a large number of asexual swimming spores each of which is capable of growing into a new individual. It was, therefore, a matter of considerable interest to investigate the sexual nature of the mycelium and spores from sprouting eggs.

Germinating eggs of *Dictyuchus* have not been observed by previous

[1] The strains of *Rhizopus* and *Phycomyces* were kindly sent us by Dr. A. F. Blakeslee.

investigators. The writer was first able to induce germination after a resting period of six weeks to two months by placing ripe eggs in boiled corn-grain juice (made by boiling three or four corn grains about five minutes in 100 c.c. water) kept in the oven at a temperature of about 28° C., approximately the optimum temperature for growth—a scheme suggested by the work of Weston (14) in germinating the eggs of *Thraustotheca clavata*, though the latter investigator used pure water in the place of a nutrient solution. Later experimentation, however, has shown that the eggs will germinate in room temperature in boiled well-water.

The method used in germinating the eggs and separating them from the surrounding threads and resting sporangia was as follows: Part of a contrast in which there were about a dozen healthy ripe eggs was cut out with a scalpel and transferred to corn-grain juice. The eggs were observed daily under the low power of the microscope, and after three or four days the first visible signs of germination became evident. The protoplasmic contents of several of the eggs had swollen considerably, enclosing the oil globule and probably increasing in size at the expense of the latter. The egg-wall had become irregular on the inner surface, apparently being resorbed, this process continuing until the egg-wall had become comparatively thin. This swelling of the egg and resorption of the oil drop continued until the egg almost entirely filled the oogonium and until the oil drop almost completely disappeared. The egg was then ready to send out a germ-tube. When an egg had sprouted a short germ-tube, it was teased out under a binocular from the rest of the material along with its own oogonial stalk and old adhering antheridial branches and transferred to a fresh drop of nutrient fluid. Here, under the binocular, with fine glass needles the antheridial threads, which, however, were apparently dead, were torn off. In some cases, however, it was impossible to separate the antheridia from the oogonia. The germinating egg was carefully observed in order to be sure that nothing but the egg was sprouting. After the germ-tube had grown into a slightly branched mycelium, the egg with its germ mycelium was either transferred to a fresh agar plate or a fresh large drop of nutrient fluid was added.

The tests of the germinating eggs fall into two classes: tests of the sexual nature of the mycelium directly descended from the sprouting egg, and tests of the sexual nature of the mycelium descended from a single spore which was descended from a sprouting egg.

Tests of the mycelium directly descended from the sprouting eggs will be described first.

Ripe eggs were cut from cross No. 33, which was made between B ♀ and B ♂ and in which oogonia were formed on November 28, and put in boiled corn-grain juice at a temperature of about 28° C., on January 21. Five days later several of the eggs had sprouted. Five of these sprouting eggs

were separated from the threads, &c., and separately planted on fresh corn-meal agar plates. The strains descended from these sprouting eggs have been designated 33 a–33 e. One of the strains, 33 b, seemed to have been injured in transference and failed to grow; another one, 33 d, gave origin to a sporangium containing seven spores, three of which germinated (see Pl. XXXVIII, Fig. 9). The sexual nature of these spores will be described later.

Two crosses were then made between each of the strains to be tested and the stock male and female strains. In none of the six crosses between the stock male strain B and the strains tested were any oogonia formed. In several of these crosses threads which had the appearance of antheridial branches were considerably developed, especially in the region between the different strains, being often apparently attracted by each other, coming in contact and apparently anastomosing in places. In all of the crosses between the stock female strain A and the strains tested oogonia were formed. In the series of crosses between A ♀ and 33 a but few oogonia were produced, while in both of the other two series of crosses between A ♀ and 33 c and A ♀ and 33 c the sexual reaction was quite strong. In the first of these last two, many oogonia were formed, the oogonia proliferating abundantly; the antheridia, however, were rather sparingly developed. In the second, A ♀ × 33 c, oogonia were produced in great abundance, many more than in other crosses, being formed over the entire female culture, but only sparingly on the sides away from 33 c. The oogonia formed on the sides farthest away from the male plant proliferated considerably, until they were reached by antheridial branches which grew directly to them for a distance of about 2 mm. The oogonia on the sides were formed several hours before any antheridia were in their vicinity; none, however, were seen which had formed eggs, whereas many of those in between the cultures, where the antheridial branches were numerous, had formed eggs. The antheridial branches were also abundantly developed, showing in a few cases the remarkable peculiarity of swelling considerably at the tip as though beginning to form an oogonial initial.

At the same time as the above eggs were sprouted and their sexual nature determined, several eggs from a cross between B ♀ and B ♂ (cross 116) were sprouted and tests were made of their sexual nature. Of several eggs which sprouted, however, only one grew (designated 116 d). Numerous crosses were made as usual with parts of the mycelium from this egg and the stock male and female strains. In all of the crosses between 116 d and A ♀ oogonia were formed, while in none of the crosses between the sprouting egg strain and B ♂ were any oogonia produced, thus indicating the male sexual nature of the mycelium from this sprouting egg.

The evidence from the above germinations and crosses seemed to indicate that sexual differentiation took place at an early stage in the egg

germination. It also has indicated, in the case of sprouting egg No. 33 c, that the sex differentiation remained constant after once being determined. The significance of this statement can be grasped only by recalling the way in which the fungus is cultured and mycelial transfers are made. Stock cultures of the fungus are kept growing on corn-meal agar plates in Petri dishes. When the mycelium has about covered the plate, as it usually does in about two weeks, it must be transferred to a fresh plate. This is done by cutting out a small cube of the agar with some of the growing ends of the fungus and planting this cube on a fresh agar plate. Hempseed cultures for crosses are inoculated in essentially the same way. It is obvious, therefore, that only a very small fraction of the mycelium is tested, and that if sexual differentiation should take place in the mycelium a good while after the egg had germinated it would easily be possible, in making the transfers, to miss one of the sexual strains entirely. To lessen the possibility of such an occurrence in the cultures of 33 c, the mycelium used for inoculation was cut from several different spots in the agar culture, and yet the crosses with A ♀ or other female strains continued to give a strong sexual reaction.

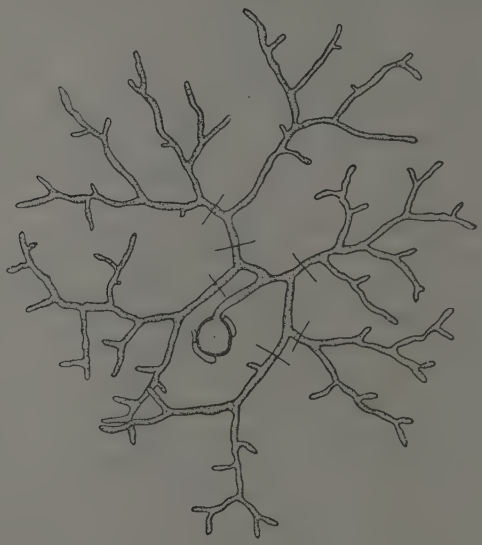
Several ripe eggs from cross 49 (A ♀ × B ♂ in corn-meal agar made on on January 14) were cut out and put in corn-grain juice February 22, and kept at room temperature, about 22° C. On February 28 the oil droplets were absorbed in most of the eggs. On March 1 fresh corn-grain juice was added. On March 2 the eggs were beginning to sprout, and on the following day the sprouts had developed into a slightly branched mycelium. Four sprouting eggs were teased out and put in fresh corn-grain juice, and were designated 49 f–i. By March 5, 10 a.m., the mycelium had grown into a loose weft of threads about 0·8 cm. in diameter, but without the formation of any sporangia. The mycelia from eggs 49 f and 49 g were transferred to separate corn-meal agar plates. The mycelia from eggs 49 h and 49 i were each divided into two parts and each part was separately planted on a corn-meal agar plate, and after being cultured for a period of ten weeks, during which time the mycelia were transferred four times to fresh plates, numerous tests were made to determine the thallic character of the mycelia. Crosses were made as usual with strains A ♀ and 33 c ♂.

The mycelia from eggs 49 f and 49 g both gave reactions which indicated their mixed sexual nature, though in the case of 49 g the reaction with A ♀ was extremely weak, only two oogonia and no antheridia being seen in four crosses. Both parts of the mycelia from sprouting egg 49 h reacted sexually in all crosses with the stock male and female strains, thus showing that sexual differentiation was not completed in any parts of the mycelia tested.

In the crosses between the mycelia from egg 49 i and the stock male and female strains strikingly interesting results were obtained. In the

tests between the mycelia from one of the parts and the stock male and female strains, mixed reactions were obtained, but in the tests with the other parts a pure male reaction was obtained, thus indicating that sexual differentiation had taken place in this part.

In another sprouting egg, designated 210 e, the germinating hyphae were allowed to develop into a considerable mat of branched threads (Text-fig. 2). This mycelium was then divided into six parts (designated M 1–6) by cutting the threads about in the position shown by the cross-lines in Text-fig. 2, and each part was then transferred to a corn-meal agar plate. After being cultured on agar for about six weeks, during which time the



TEXT-FIG. 2. Sprouting egg 210 e.

fungus was transferred four times to fresh agar plates, cultures were made from all six of the mycelia and crossed in the usual way with the stock male and female strains. In these tests one of the parts of the mycelium gave a pure female reaction in all crosses, while all of the five other parts gave a mixed reaction.

These crosses brought to light several points of interest. The developing mycelium may become differentiated into several areas, certain of which may be apparently pure male, others pure female, and still other areas in which the two sexes are still undifferentiated, the segregation of the sex-determining substance taking place without the formation of any sporangia. In the three sets of crosses which produced a mixed reaction, the crosses between the stock male strain B and M 1, M 5, and M 6 pro-

duced many normal oogonia and antheridia, excepting one of the two crosses between B ♂ and M 6, in which no observable reaction took place. In the crosses between the stock female strain A and M 1, M 5, and M 6 only oogonial initials were formed, the oogonia usually proliferating considerably and becoming nearly empty of protoplasm, no antheridia being developed. It seems probable that in these latter crosses the mycelia of strains M 1, M 5, and M 6 contained both sexual natures, and that while the male nature was strong enough to stimulate the female to the formation of oogonial initials, it was not strong enough to develop antheridia to complete the formation of the sexual reproductive bodies. The reaction which B ♂ gave with M 1, M 2, M 5, and M 6 was quite remarkable and consistent with results formerly obtained in crosses between B ♀ and B ♂. In these crosses the antheridial strain was greatly stimulated, so that many of the oogonia were completely encircled with antheridia. Practically all the oogonia were furnished with antheridia, and a large majority of them were developing eggs. The very small number of the oogonia which were without antheridia had exhausted their contents by repeated proliferation.

The crosses between M 3 and B ♂, and M 4 and B ♂, showed no sexual reaction when first examined, while at the same time the crosses between A ♀ and M 3 and M 4 produced fairly strong reactions, though M 4 formed no antheridia. It was concluded, therefore, that M 3 and M 4 were antheridial strains. Upon a re-examination of the former crosses a week later, a few oogonia and antheridia were found in them, and therefore the mycelium in these strains, M 3 and M 4, had to be considered as of a mixed sexual nature. The crosses of the strain M 2, which was giving a female reaction, were also re-examined, but no oogonia or antheridia have been formed in the contrasts with strain A ♀. Strain M 2 seemed, therefore, to be female.

It seemed from the results obtained in the mycelial tests that sexual differentiation might take place in the mycelium, and that certain regions might be male and other regions female in nature, but that, due to the fact that as growth proceeds the threads of both sexes become intermixed, it usually happens in making tests that a mixed reaction results, since a good many threads are usually cut out with each agar square. In view of the results of Blakeslee (1) with *Mucor mucedo*, in which he found that the segregation of sex is completed at some time before the formation of sporangial spores, and that all the spores in a given germ sporangium were of the same strain, it seemed a matter of no small import to induce the early formation of sporangia in the germinating egg, and to test the sexual reactions of the spores. The germinating egg of *Dictyuchus*, as has already been seen above, sprouts into a germ-tube which soon branches into a mycelium. This mycelium, if kept in a liquid medium, usually

forms numerous sporangia. If the germ mycelium is transferred to practically pure water, the early formation of sporangia may be readily induced.

One of the germinating eggs, 33 d, from cross 33 sprouted into a germ-tube which, without branching, bore a single sporangium containing seven spores, three of which germinated as has been noted above (see Pl. XXXVIII, Fig. 9). These three spores were teased out with fine glass needles, and planted separately on corn-meal agar plates. The mycelia from these spores were then tested with the stock male and female strains. In four tests between cultures descended from each of the spores and the stock male strain B no reaction was obtained; while in the same number of tests with the female strain A oogonia and antheridia were formed in all of the crosses, thus proving the three spores to be antheridial in their sexual nature. Unfortunately, though several other efforts were made, no more eggs could be induced to germinate in this manner.

The mycelium of one of the five sprouting eggs, namely 49 j, from cross 49, tests of four of which have been discussed above, was transferred to sterile well-water on March 5. On the following day many sporangia had been formed. The early sporangia on the mycelium of a germinating egg are usually rather small, containing from eight to about fifty spores. One small sporangium containing about fifteen spores was separated out from the mycelium of 49 j and placed in a fresh drop of corn-grain juice. After about six hours the spores had sprouted, the germ-tubes growing out through the sporangial wall. The sporangium was then picked up with a glass needle with a knob on the end of it and transferred to a fresh agar plate, and there, under a low-power binocular, with very fine glass needles the sprouting spores were separated from each other, picked up on the end of a very fine glass needle with a small knob on the end, and placed on fresh agar plates (March 8, p.m.). After twenty-four hours eleven of the spores had developed a slightly branched mycelium. These eleven were designated j 1–11.

In order to ascertain the sex character of each of the eleven mycelia, several crosses were made between each of the eleven spores and strains A ♀ and B ♂ and 33 c ♂. In these crosses five of the eleven spores produced male mycelium, one female, four produced mixed mycelium, and one spore a neutral mycelium. Only two of the spore strains produced any reaction with B ♂, while of the six crosses between the spore strains and 33 c ♂ four produced oogonia and antheridia, thus indicating again the strong male nature of 33 c and the relatively weak male nature of B ♂.

Fifteen spores from another sporangium were isolated and tested as above. The strains descended from the sprouting spores were designated k 1–15. In these tests no pure males were found, while five spores produced mycelium which was female in nature, nine produced mycelium

which reacted with both the antheridial and oogonial strain, and one produced a mycelium neutral in its reactions.

Of the eleven spores tested in the first series, four produced antheridia when crossed with A ♀, and oogonia when crossed with 33 c ♂ or B ♂, and of the fifteen tested in the second series nine reacted both with the antheridial and oogonial strains. In most of the spores which produced a mixed mycelium the reaction was either considerably stronger male than female, or vice versa. As shown from the results with the fifteen spores in the second series, the four mixed mycelia gave a stronger reaction with the antheridial strain, 33 c, than with the oogonial strain A. In the crosses with the mixed mycelia, which produced a weak sexual reaction, the sexual organs were usually limited to a small area in the crosses, generally only a few threads bearing oogonia and a few bearing antheridia, the greater part of the threads, even where the two opposing strains were in contact, remaining sexually inactive. In spite of the fact that such mixed strains, when kept separated from strong oogonial or antheridal strains, have invariably failed to produce any sexual reproductive bodies, the production of such strains from a single spore capable of reacting with both the oogonial and antheridial strains would seem to indicate that such a mycelium was homothallic in nature.

It was a matter of interest to find out if the mycelia from these apparently homothallic spores continued to be homothallic after being cultured for a month or more, and if the mycelia from spores which gave a pure male or pure female reaction in the first tests would continue to do so. The mycelia from the spores k 1–15 were kept growing on corn-meal agar plates, and about six weeks after the first tests were made another series was made in the same way. In comparing the results in the two sets of crosses it is seen: that in the first series there were no pure males, while in the second two spore strains, k 9 and k 11, gave strong male reactions; that two of the five strains giving a pure female reaction in the first set gave a mixed reaction in the second set; that the strain which gave a neutral reaction in the first cross gave a mixed reaction in the second series; and that the number of mixed strains remained relatively the same.

The tests seem to indicate that the sex may be segregated in the early stages of egg germination, or that it may take place in the early formed sporangia, but that in these sporangia the segregation is only partial, as some of the spores give rise to mycelia which might be considered homothallic. Interesting in this connexion are the results obtained by Blakeslee (2) with *Phycomyces nitens*, in which he found that a segregation of sex may take place at the formation of spores in the germ sporangia, which, however, is only partial, as, in addition to (+) and (−) heterothallic spores, spores are formed which give rise to homothallic mycelia characterized by a production of contorted aerial outgrowths termed pseudophores and the

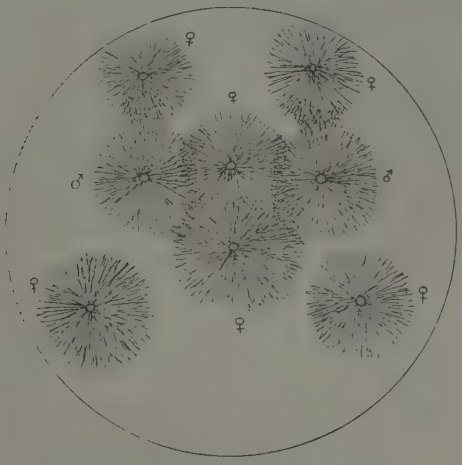
occasional formation of homothallic zygospores. He also reports that the sexual character in these homothallic mycelia is unstable, and in their sporangia a segregation again takes place and (+), (−), and homothallic spores are produced. The results obtained in the tests with germinating eggs in *Dictyuchus* furnish a striking parallel to those obtained by Blakeslee in *Phycomyces*.

Morphology and Physiology of Reproduction.

In species of the Saprolegniaceae in which sexual reproduction apparently takes place the sexual organs are of two very distinct morphological types, namely, the oogonia, which are usually comparatively large, and the antheridia, which are much smaller. In all species which have been carefully studied the oogonial initials appear first, the antheridial branches appearing later and growing to the oogonia. The oogonia, so far as observation has gone, do not seem to be attracted by the antheridia, but there seems to be a strong attraction on the part of the oogonia for the antheridia, as the latter quite often grow to the oogonia from a considerable distance. In species in which fertilization has been proved, as *Achlya de Baryana* by Trow (13) and *Saprolegnia monoica* by Claussen (3), antheridia are applied to the oogonial wall, antheridial cells are cut off, and part of the contents of the male gametangium is discharged into the developing egg.

In *Dictyuchus* the oogonial and antheridial strains are indistinguishable from each other when grown separately. The threads are all rather thick, only slightly branched, and bear numerous sporangia. If the two sexual strains are placed so that their threads are in contact, the two strains are considerably stimulated, finally resulting in the production of numerous sexual reproductive bodies. The stimulus often first manifests itself by more or less renewed growth, especially on the sides of the cultures where the threads are in contact. Usually, and especially in cultures which produce a strong reaction, numerous hyphae grow out from the substrata of the opposite cultures, these threads intermingling, often apparently anastomosing in places, and finally forming a dense mat of hyphae, those from the oogonial culture forming numerous oogonial initials, and those from the antheridial culture branching out into antheridial threads. Quite often, however, the oogonial and antheridial initials may arise from the primary threads. As is the case with monoecious species of the Saprolegniaceae, the oogonial initials are formed several hours before the antheridia come in contact with them. Oogonial initials may be formed on the side of the culture on threads which are not in contact with the threads from the antheridial culture. In some cases antheridial branches have been observed to grow a distance of two millimetres to such oogonia, in which case the oogonia formed eggs.

The question now arises as to the nature of the stimulus which causes the formation of the reproductive bodies. It seemed logical to assume that some substance was given off by the male plant which diffused through the water and stimulated the female in such a way that it formed oogonia. To test this hypothesis a female plant was put in the same Petri dish with a male, but placed so that the threads of the two plants were not in contact. After several days the two cultures were examined, but no observable change had taken place in either plant. This experiment has been repeated many times with the same result. In view of the possibility that the sexual



TEXT-FIG. 3. For explanation see text.

substance given off by one culture only might diffuse throughout the liquid and become too weak to cause any stimulation, a female strain was put into the dish with four young healthy antheridial cultures without changing the water in which the antheridial strains were growing, but still no oogonial initials were formed, nor were the antheridial strains in any recognizable way affected. Eight antheridial cultures were now used, but with the same result.

It might be that the sexual substance is given off only when the threads of the opposite strains are in contact. That such might be the case seemed to be suggested by the occurrence of oogonial initials out on the side of the female culture a considerable distance away from the region where the threads of the two opposite strains were in contact. To test the possibility of a stimulating substance being given off under such a condition, crosses were made between two female cultures and two male cultures as shown in the diagram, Text-fig. 3, and at the same time four oogonial cultures growing on bits of boiled corn grain were placed in the dish, two with their

threads barely crossed over those of the antheridial strain and two placed away from the antheridial strain a distance of about 4 mm. Upon examination several days later, many oogonia and antheridia were formed on the cultures in contact in the regions where the threads of the strains of opposite sex intermingled. Oogonia were also formed on the two oogonial cultures the threads of which were barely in contact with those of the antheridial strain. The threads of the antheridial strains had grown considerably, becoming so intimately associated with those of the latter two oogonial strains that even when the cultures were washed with a strong stream of water from a wash-bottle they were not separated. On the two oogonial cultures which were not placed in contact with the antheridial strains no oogonia were formed. This experiment has been repeated several times with the same results.

Other experiments have been made in which the two opposing strains were crossed as usual, but with a very thin collodion membrane separating the threads, so that the threads of the two strains could not come in contact and anastomose. In these experiments the female threads in the region covered by the collodion membrane became unhealthy in a large percentage of the cultures, due probably to a poor oxygen supply, but even in the cultures in which the covered threads remained healthy no sexual reaction occurred.

To obviate the possibility of a poor oxygen supply interfering with the reaction the following scheme was used: The male was placed on one side of a collodion membrane and the female on the opposite side, and then this was placed over a tall glass ring with the female on the under side of the membrane in a Petri dish filled with enough water to leave an air-space about 1 mm. deep in the ring. The sagging of the membrane made a nice bowl to contain water and thus prevent the male culture from drying, and at the same time pushed the hempseed on which the female was growing far enough into the water to keep that culture wet. No reaction was obtained, however, in any of these crosses. To obtain a still greater oxygen supply, bowl-shaped collodion membranes were used. With the male in about a centimetre of water on the inside of the bowl and the female opposite the male on the outer and under side, the bowl was glued to the top of the Petri dish chamber, which was filled with enough water to partially immerse the female culture. But in these tests no reaction was obtained.

Hard filter-paper was now used in place of the collodion membranes. Upon examining the cultures several days after they were made, many oogonia were found on the female culture. Both cultures stuck tenaciously to the filter-paper, indicating almost certainly that some of the threads had grown through the paper. Moreover, some of the oogonia had antheridia on them, and one oogonial initial was seen on the male side, but the thread

bearing it was not connected with the male strain. Essentially the same results were obtained in several similar experiments.

It seems, therefore, from the data so far presented, that the stimulus initiating the sexual reaction passes through the anastomoses in the coenocytic mycelium.

An experiment was now carried out to determine if the potency of the active substance in the male which stimulated the female to the formation of oogonial initials was lost if the male was crushed and the female put in the expressed, filtered juice from the male. Eight young healthy cultures of the antheridial strain were crushed with a mortar and pestle and filtered with about 10 c.c. of water through a suction filter. A healthy young female was now put on a sterilized hollow-ground slide and the juice from the male poured over it. Upon examination several days later, the female was found to be quite healthy, but showed no sexual stimulation. This experiment was repeated several times with similar results. Other tests were carried out in which the juice from the males was further diluted, but no sexual stimulation was observed in the female. Antheridial strains were also put in juice expressed from oogonial strains, but no observable stimulation took place.

It seemed from these experiments that in order to stimulate the female to the formation of oogonial initials it was necessary to have a living healthy male actually in contact with the female culture.

To find out just how much of a male culture was required to stimulate the female to the formation of oogonial initials, the following tests were made: A single healthy thread was cut from a male culture and placed across the threads of a healthy female culture a couple of millimetres behind their tips. The male thread healed up at the cut end and remained apparently healthy, but produced no stimulation in the female. This experiment was repeated several times, using one male thread, two, and three respectively, but all with the same results. Six threads were cut from the same male culture from which the threads used in the above experiments were cut, and placed across the threads of a female culture. When examined several days later, a considerable number of oogonia had formed on the female threads in the region where the male threads were in contact, and a few antheridial branches had grown out from the male threads, applying themselves to the oogonia.

Although no systematic attempt has been made to determine the effects of varying the external conditions on the formation of sexual reproductive bodies, yet it has been repeatedly noticed that alterations in the temperature or food material produced variations in sexual activity. It has been found that crosses between certain strains produced oogonia more readily and in greater abundance when kept at a temperature about 26° C., while in crosses between most strains the optimum temperature for the

formation of oogonia and antheridia seemed to be around 22° C. Two kinds of seeds have been used in the cultures more extensively than any others, namely, hempseed and corn grain. While the former has a particular advantage over the latter in that cultures on it are relatively free from bacteria, still cultures of opposite strains grown and crossed on the latter, if the bacteria can be kept out, produce oogonia and antheridia much more readily than cultures on the former. Moreover, considerable variation has been found to occur in sexual activity as the concentration of the food material was changed. Crosses of opposite strains on corn-meal agar made up according to our usual formula produced no sexual stimulus, while if the amount of the corn meal was doubled, as seen above, oogonia and antheridia were formed. It may be concluded here, as Blakeslee (1) did in the case of *Mucor mucedo*, &c., that variations in the external conditions have a secondary and variable effect in influencing sexual reproduction.

Summary.

The strains of *Dictyuchus* as collected in nature show certain variations which, according to the general principles of classification in this group, would permit the making of several species, as has already been done.

In strains which were producing sexual fruits the antheridial and oogonial strains have been isolated and grown separately, under which conditions they invariably remain sexually sterile, but when the opposite strains are grown together so that their threads intermingle, oogonia and antheridia are formed in the region where the threads are in contact, thus proving the plant to be dioecious or heterothallic.

To date, six female strains, nine male, and eleven neutral strains have been collected.

The opposite strains, with a few exceptions, of the apparently different species may be intercrossed, in which crosses variations occur in the size of the oogonia and eggs and in the character of the antheridia which seem to invalidate any specific distinctions between the species *D. monosporus*, *D. Magnusii*, *D. carpophorus*, and *D. sterile*.

A heretofore unreported strain which forms oogonia and eggs without antheridia is described. In crosses between strains descended from the germinated parthenogenetic eggs and the male strain 33 c, the male strain, in a few cases, was stimulated to the formation of antheridia which applied themselves to the walls of the oogonia of the parthenogenetic strain. In 2 out of 98 crosses between the strains descended from the germinated parthenogenetic eggs and the female strain A oogonia were formed on the latter as well as on the parthenogenetic strain, and the parthenogenetic strain was stimulated to the formation of antheridia which applied them-

selves to the oogonia of strain A ♀; in which cases, as a rule, eggs were formed.

In numerous crosses between the male and female strains of *Dictyuchus* and other moulds sexual stimulation occurred only between the female strain A of *Dictyuchus* and *Thraustotheca primoachlya*, in which attempts the *Dictyuchus* formed oogonial initials and the *Thraustotheca* formed a superabundance of antheridial branches, the stimulation occurring only in the region where the threads of the two plants intermingled.

The eggs of *Dictyuchus*, after a rest period of a month to six weeks, become capable of germinating either into a short hypha which bears a single sporangium, or into a more or less branched mycelium.

Numerous eggs have been germinated and considerable experimentation has been done to find out when sexual segregation took place. It was found that partial sexual segregation may take place early in the egg germination, so that parts of the mycelium may be male, parts female, and parts may be mixed, or that segregation may take place at the formation of spores in the early formed sporangia, in which some of the spores may be male, part female, and part mixed.

Considerable experimentation has been carried on to determine the nature of the stimulus which causes the formation of the reproductive bodies. It has been found that the reproductive bodies are formed only when the male and female strains are actually in contact. A female culture placed in the same dish with as many as eight male cultures, but not in contact with any of them, was unaffected by the proximity. Cultures of the opposite sex crossed as usual, but with a collodion membrane between, gave no reaction; if, however, a hard filter-paper is placed between the two cultures, the threads grow into the paper, come in contact, and oogonia and antheridia are formed. Oogonial strains put in the juice expressed from antheridial cultures were apparently unaffected. If, however, as few as six threads are cut from the antheridial culture and placed across the threads of an oogonial culture, sexual stimulation is obtained in this region.

No extensive attempt has been made to determine the effects of external conditions on the formation of sexual reproductive bodies, yet it may be concluded that variations in the external conditions have a secondary and variable effect in influencing sexual reproduction.

Literature cited.

1. Blakeslee, A. F.: Sexual Reproduction in the Mucorineae. Proc. Amer. Acad. Arts and Sciences, xl. 205–319, 1904.
2. ———————: Zygospore Germinations in the Mucorineae. Annales Mycologici, iv. 1–28, 1906.
3. Claussen, P.: Über Entwicklung und Befruchtung bei *Saprolegnia monoica*. Festschr. d. Deutsch. Bot. Gesell., xxvi. 144, 1908.
4. Coker, W. C.: The Saprolegniaceae. The Univ. of North Carolina Press, Chapel Hill, N. C. 1923.
5. Fischer, A.: Saprolegniineae. Rabenhorst's Krypt.-Fl., i, Abt. 4, 310, 1892.
6. Humphrey, J. E.: The Saprolegniaceae of the U.S., &c. Trans. Amer. Phil. Soc., xvii. 63, 1893.
7. Kauffman, C. H.: A Contribution to the Physiology of the Saprolegniaceae, with Special Reference to the Variation of the Sexual Organs. Ann. Bot., xxii. 361, 1908.
8. Leitgeb, H.: Neue Saprolegnieen. Jahrb. f. wiss. Bot., vii. 357, Pls. XXII–IV, 1869–70.
9. Lindstedt, K.: Synopsis der Saprolegniaceen und Beobachtungen über einige Arten, p. 69, Pl. 4. Berlin, 1872.
10. Minden, M. von: Saprolegniineae, in Kryptogamenflora der Mark Brandenburg, v. 209–600, 1912.
11. Pieters, A. J.: The Relation between Vegetative Vigor and Reproduction in some Saprolegniaceae. Amer. Journ. of Bot., ii. 529, 1916.
12. Tiesenhausen, M.: Beiträge zur Kenntnis der Wasserpilze der Schweiz. Archiv f. Hydrobiol. u. Planktonkunde, vii. 261–308, 1912.
13. Trow, A. H.: On Fertilization in the Saprolegniaceae. Ann. Bot., xviii. 541, 1904.
14. Weston, W. H.: The Development of *Thraustotheca*, a Peculiar Water Mold. Ann. Bot., xxxii. 155, 1918.
15. ———————: Repeated Zoospore Emergence in *Dictyuchus*. Bot. Gaz., lxviii. 287, Pl. XXIII, 1919.
16. Zopf, W.: Über eine Saprolegniee mit einer Art von erysipheenähnlicher Fruchtbildung. Beiträge z. Physiol. u. Morphol. d. nied. Org., iii. 48–59, Pls. II, III, 1893.

EXPLANATION OF PLATES XXXV–XXXVIII.

Illustrating Dr. J. N. Couch's paper on Heterothallism in *Dictyuchus*.

PLATE XXXV.

The upper photograph is of a cross between the female strain A and the male strain B, showing the thick region of oogonia and antheridia where the threads of the two strains intermingle. Stained with iron-alum haematoxylin. × 5.

The photomicrograph below shows an enlarged view of the region producing sexual organs in the above cross. The oogonia are the spherical or slightly sub-spherical objects. The antheridia, applied to the oogonia, are visible in some cases. None of the oogonia has yet formed eggs. The dark elongated bodies are sporangia. × 25.

PLATE XXXVI.

Photomicrograph of the edge of a cross showing oogonial threads extending from the left bottom corner towards the top right corner, whereas the antheridial threads are coming to the oogonia from the upper left corner. Numerous sporangia are shown. The small circles are resting spores and spore cysts. × 100.

PLATE XXXVII.

Fig. 1. Sporangia borne in sympodia. Strain B.

Figs. 2, 3. Sporangia borne in rows. Strain I.

Fig. 4. Sporangium showing emerging spores. Strain C.

Fig. 5. Sporangium of the *Thraustotheca* type. Strain G.

Figs. 6, 7, 8. Habit sketch of oogonia, antheridia, and sporangia from the original culture of strain B.

Figs. 1, 2, 3, × 90. Figs. 4, 5, × 250. Figs. 6, 7, × 670. Fig. 8, × 250.

PLATE XXXVIII.

Fig. 1. Ripe egg of *Dictyuchus* showing eccentric oil drop partially buried in the larger mass of protoplasm.

Fig. 2. Egg beginning to germinate; the oil drop surrounded by protoplasm.

Fig. 3. Oil drop being resorbed, showing a frayed outline. Egg-wall being resorbed, also showing an irregular outline. Cytoplasm granular.

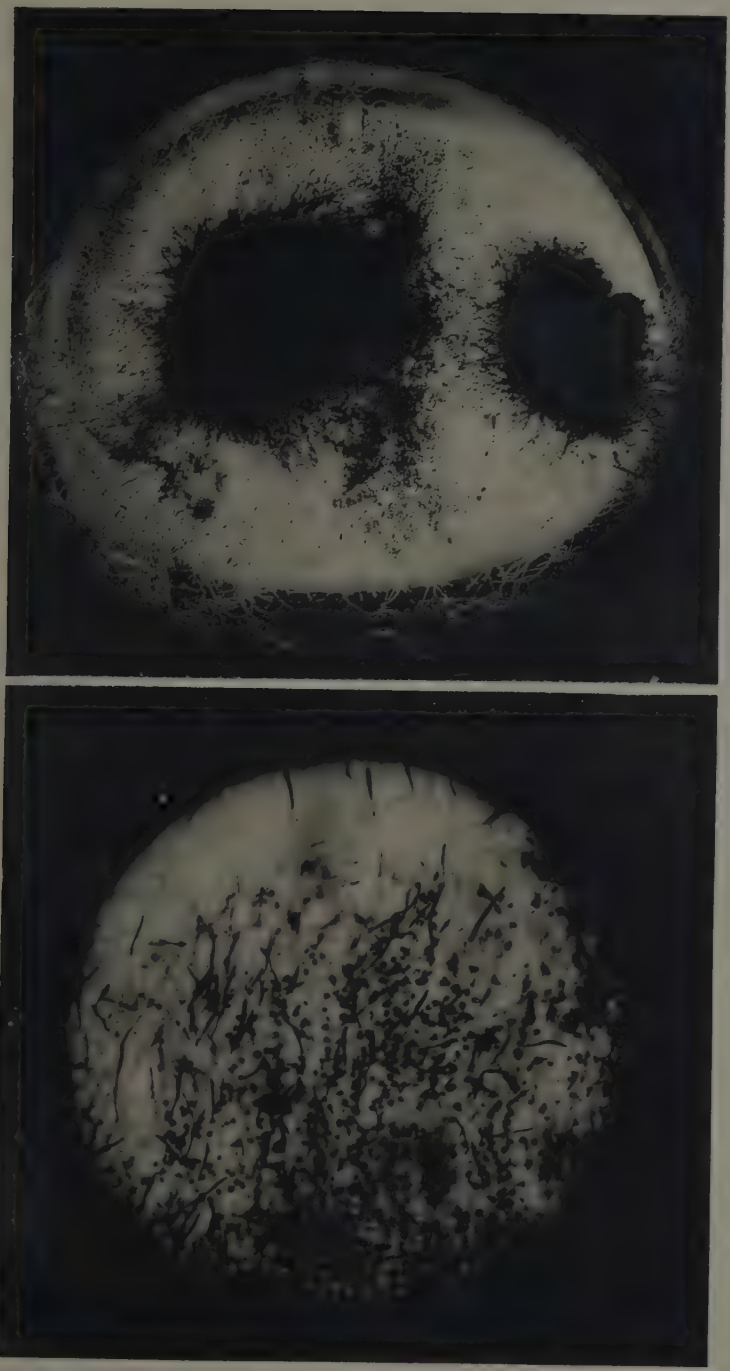
Figs. 4, 5, 6, 7. Progressive stages in sprouting eggs, showing diminution in the oil drop, thinning of the egg-wall, and swelling of the egg.

Fig. 8. Egg germinating into a hypha.

Fig. 9. Germ-tube which contained seven spores, three of which have germinated. The sexual nature of these three germinated spores was tested, as described in the text, and all three were found to be male in nature.

Fig. 10. Showing a part of the edge of the region where the threads of A ♀ and B ♂ were intermingling, the oogonial threads coming from the upper right edge of figure, the antheridial threads coming from the bottom of the plate. A few oogonia are not furnished with antheridia, but are forming eggs.

Figs. 1–9, × 620. Fig. 10, × 162.

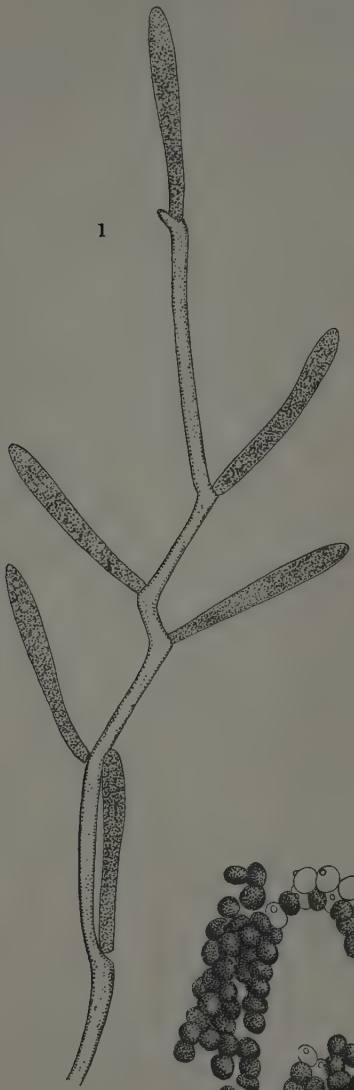


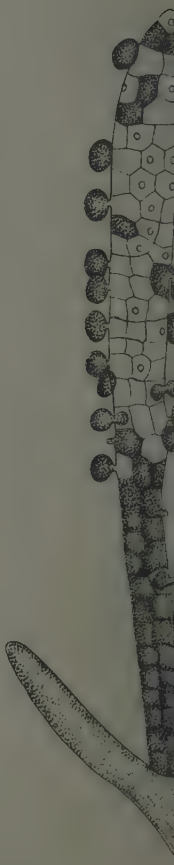
Huth coll

COUCH — HETEROTHALLISM IN DICTYUCHUS.

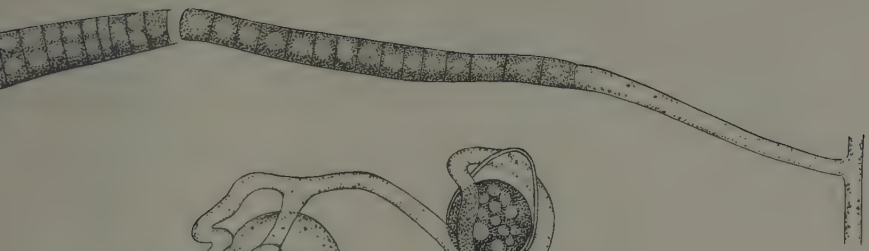
Huth coll.

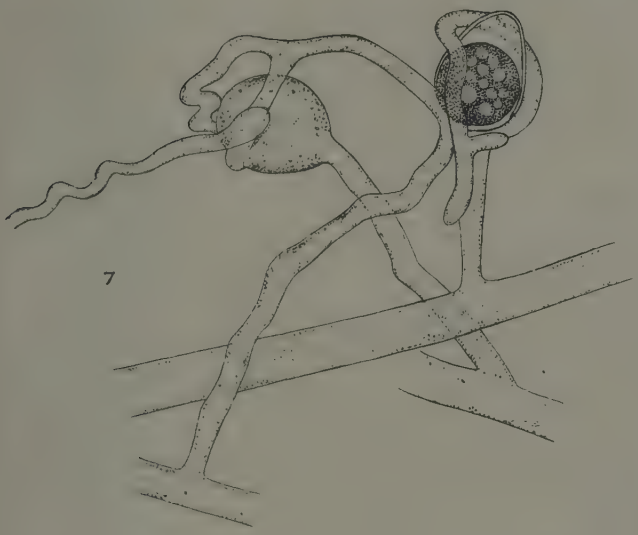
COUCH—HETEROTHALLISM IN DICTYUCHUS.



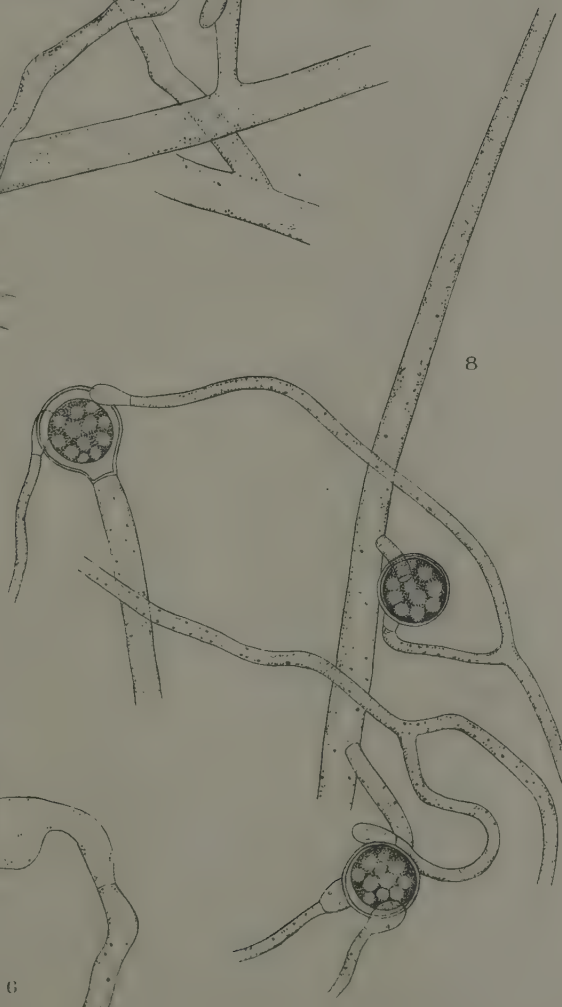


COUCH—HETEROTHALLISM IN DICTYUCHUS.





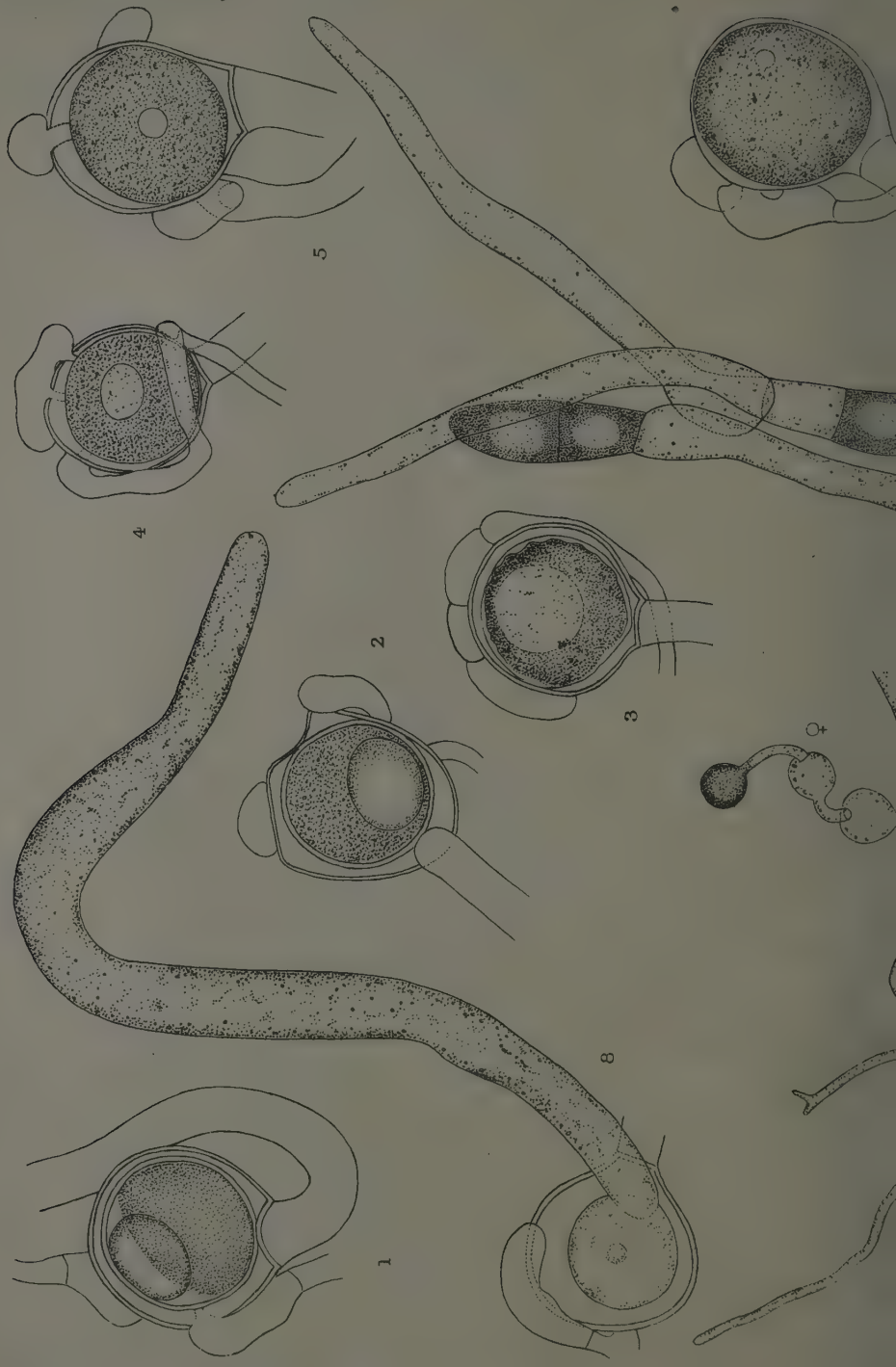
7



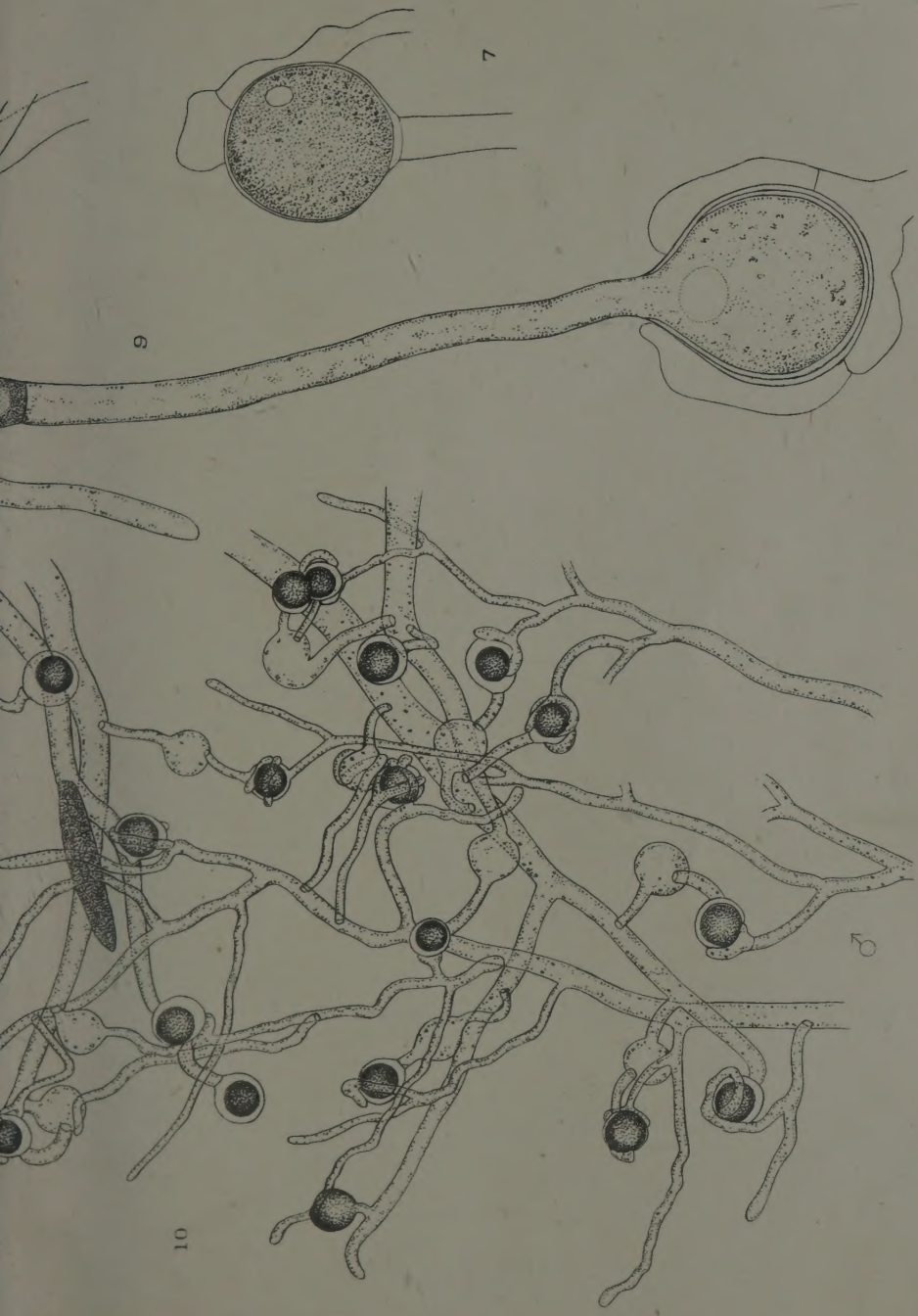
8

6

Huth, London.



COUCH—HETEROTHALLISM IN DICTYUCHUS.



Huth, London.